

Appln. No. 10/574,197  
Amdt. dated September 17, 2009  
In reply to the Office Action of March 17, 2009

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1-12. (Cancelled)

13. (Currently amended) Method ~~A method~~ for the continuous, semi-continuous or discontinuous treatment of a substrate (24), said method comprising:

~~(a) in which introducing said substrate (24) into installed in a bioreactor vessel (1) is subjected to the action of a culture of~~ containing various populations of living cells C1 making it possible to carry out a reaction R1 on capable of carrying out a bioconversion of said substrate (24), characterized in that it comprises the following steps:

~~(a)~~ (b) periodically sampling and transferring a sub-population of the removing a population of living cells C1 present in the substrate in the bioreactor vessel (1) in order to transfer them into an automatic device (2) for selecting living cells proliferating in suspension;

~~b)~~ (c) selecting the cells C2 proliferating in suspension under sterile conditions in said device (2), by eliminating the static living cells, and;

~~e)~~ (d) transferring all or part of the selected cells C2 proliferating in suspension back to the non-sterile

~~present in said selection device (2) periodically to the~~  
bioreactor vessel (1) and mixing said cells C2 with the living  
cells C1 present within said substrate; and

(e) repeating steps (b) to (d), whereby the  
mixing of the living cells C1 and the cells C2 results in  
bioconversion of the substrate in the bioreactor vessel (1).

**14. (Currently amended) Method**~~The method~~ according  
to claim 13, ~~characterized in that wherein~~ the automatic  
device (2) for selecting cells proliferating in suspension  
comprises:

- two or more vessels (20, 21) ~~making it possible~~configured  
to receive and maintain cultures of living cells in  
suspension,
- a set of means making it possible to separately supply  
~~these the two or more~~ vessels with sterilizing (25),  
cleaning or neutralizing fluids,
- a set of means making it possible to supply ~~these the two~~  
or more vessels with gas (23),
- a set of means making it possible to supply ~~these the two~~  
or more vessels with ~~substrate (24)~~sterile culture medium  
(24),
- a set of means (28-31) making it possible to transfer the  
content of one vessel (20) into the other (21) and vice-  
versa,

- a set of means making it possible to evacuate all or part of the content of ~~these~~ the two or more vessels to ~~another device such as a~~ the bioreactor vessel (1),
- a set of means making it possible to evacuate all or part of the content of ~~these~~ the two or more vessels (20, 21) to a refuse bin.

**15. (Currently amended) Method** ~~The method~~ according to claim 13, ~~characterized in that~~ wherein the automatic device for selecting cells proliferating in suspension comprises ~~in particular:~~

- (a) at least one first and at least one second culture vessel (20, 21) ~~intended~~ adapted to receive a culture (22),
- (b) a source of gas (23);
- (c) a source of medium ~~(substrate)~~ (24);
- (d) a source (25) for a sterilizing agent; and
- (e) a system of pipes comprising means for connecting either one of the two culture vessels (20 or 21) to the source of the medium (24) ~~such as valves~~ as well as for connecting the two culture vessels (20, 21) to each other and for connecting either of the other culture vessels (20 or 21) to the source (25) of the sterilizing agent.

**16. (Currently amended) Method** ~~The method~~ according to claim 13, ~~characterized in that~~ wherein the living cells C2

Appln. No. 10/574,197  
Amdt. dated September 17, 2009  
In reply to the Office Action of March 17, 2009

originate from the selection carried out from a population of living cells proliferating exclusively in suspension.

17. **(Currently amended) Method** ~~The method~~ according to claim 13, ~~characterized in that~~ wherein the bioreactor vessel (1) is chosen from an aeration tank of a treatment plant, a methanization tank, an anaerobic biological treatment unit, a lagoon, a reservoir, a tank ~~for example~~ from 0.5 litre to 100 m<sup>3</sup> or a fermenter.

18. **(Currently amended) Method** ~~The method~~ according to claim 13, ~~characterized in that~~ wherein the living cells C2 proliferating in suspension can ~~in particular~~ be produced by implementation of a method comprising the following stages:

- (a) making available a culture (22) in at least one first culture vessel (20);
- (b) continuous supplying of the culture (22) in the first culture vessel (20) with gas from a source of gas (23) and regular replenishment with ~~liquids from a source of substrate~~ sterile culture medium (24),
- (c) transfer of the culture (22) from the first culture vessel (20) by connecting pipes (28-31) into at least one second culture vessel (21) by means of an appropriate pipe circuit,

Appln. No. 10/574,197

Amdt. dated September 17, 2009

In reply to the Office Action of March 17, 2009

- (d) connection of the first culture vessel (20) to a source (25) for a sterilizing agent, in order to sterilize the first culture vessel (20),
- (e) removal of the sterilizing agent from the first culture vessel (20),
- (f) continuous supplying of the culture (22) in the second culture vessel (21) with gas from the source of gas (23) and regular replenishment with liquids from the source of sterile culture medium (24),
- (g) return of the culture (22) from the second culture vessel (21) via the connecting pipes (28-31) into the first culture vessel (20) by means of an appropriate pipe circuit,
- (h) connection of the second culture vessel (21) to the source (25) for the sterilizing agent, in order to sterilize the second culture vessel (21); and
- (i) removal of the sterilizing agent from the second culture vessel (21).

19. **(Currently amended) Method** ~~The method~~ according to claim 13, ~~characterized in that~~ wherein the substrate (24) is chosen from a ~~medium containing a compound the metabolic conversion of which is envisaged,~~ water of industrial origin, domestic sewage, an accidental pollutant of the environment ~~such as the presence in the sea of a slick of hydrocarbons or~~

Appln. No. 10/574,197

Amdt. dated September 17, 2009

In reply to the Office Action of March 17, 2009

~~ether chemical products~~, chemical effluents spread on the ground, soil polluted with heavy metals or dioxin, a compound the metabolic conversion of which is envisaged ~~such as~~ ~~glucose, ethanol or oxalic acid~~, a volatile organochlorinated compound, an organochlorinated pesticide, a halogenated polycyclic aromatic hydrocarbon or a solvent.

20. (Currently amended) ~~Method~~ The method according to claim 13, ~~characterized in that~~ wherein the living cells comprise one or more species chosen from bacteria, animal or plant cells, cells of algae, yeasts or fungi.

21. (Currently amended) ~~Method~~ The method according to claim 13, ~~characterized in that~~ wherein the transfer originating from the automatic device (2) for selecting living cells ~~envisaged in stage e)~~ (d) is carried out at least once a week.

22. (Withdrawn) Device for culturing living cells characterized in that it comprises:

- A: a bioreactor vessel (1) capable of containing a population of living cells C1 carrying out a reaction R1 of bioconversion of a substrate (24);
- B: an automatic device (2) for selecting living cells proliferating in suspension, making it possible to select

Appln. No. 10/574,197

Amdt. dated September 17, 2009

In reply to the Office Action of March 17, 2009

living cells C2 proliferating in suspension by elimination of the static living cells, this device comprising a set of means making it possible to supply of said device with sterilization, washing or neutralizing liquids;

- C: a system of pipes (5) comprising means for carrying out transfers from the selection device (2) to the bioreactor vessel (1); and
- D: a system of pipes (6) comprising means for carrying out transfers from the bioreactor vessel (1) to the selection device (2).

23. (Withdrawn) Device for culturing living cells according to claim 22, characterized in that it comprises, in addition:

- E: a pipe (15) comprising means for connecting the bioreactor vessel (1) to a solid-liquid separation device such as a settling tank (16).

24. (Withdrawn) Device for culturing living cells according to claim 22, characterized in that the selection device (2) comprises:

- two or more vessels (20, 21) making it possible to receive and maintain cultures of living cells in suspension,
- a set of means making it possible to supply these vessels with substrate (24),

Appln. No. 10/574,197

Amdt. dated September 17, 2009

In reply to the Office Action of March 17, 2009

- a set of means (28-31) making it possible to transfer the content of one vessel (20) into the other (21) and vice-versa,
- a set of means making it possible to evacuate all or part of the content of these vessels to another device such as a bioreactor vessel (1),
- a set of means making it possible to evacuate all or part of the content of these vessels (20, 21) to a refuse bin.

25. (Withdrawn) Device for culturing living cells according to claim 22, characterized in that the device for selecting living cells proliferating in suspension comprises:

- (a) at least one first and at least one second culture vessel (20, 21) intended to receive a culture (22),
- (b) a source of gas (23),
- (c) a source of medium (2),
- (d) a source (25) for a sterilizing agent; and
- (e) a system of pipes comprising means for connecting either one of the two culture vessels (20 or 21) to the source of medium (24) such as valves as well as connecting the two culture vessels (20, 21) to each other and for connecting either one of the other culture vessels (20 or 21) to the source (25) of the sterilizing agent.



Appln. No. 10/574,197  
Amdt. dated September 17, 2009  
In reply to the Office Action of March 17, 2009

**26. (Withdrawn)** Device according to claim 22, characterized in that the bioreactor vessel (1) is chosen from an aeration tank of a treatment plant, a methanization tank of an anaerobic biological treatment unit, a lagoon, a reservoir or a tank from 0.5 litre to 100 m<sup>3</sup>.

**27. (New)** The method of claim 15, wherein the means for connecting either one of the two culture vessels (20 or 21) to the source of the medium (24) comprises one or more valves.

**28. (New)** The method of claim 19, wherein:  
the accidental pollutant of the environment is selected from the group consisting of the presence in the sea of a slick of hydrocarbons and other chemical products, and  
the compound the metabolic conversion of which is envisaged is selected from the group consisting of glucose, ethanol and oxalic acid.